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Electronic Structure and Cytotoxic Activity of "Half-Mustard Type" Phenothiazines by MM3 and PM3 Methods

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Abstract. *Among 54 cancer cell lines, colon-cancer cells were the most sensitive to six "half-mustard type" phenothiazines [**7-12**], followed by leukemia, melanoma, prostate-, CNS-, breast-, lung-, renal and ovarian cancer cells. The distribution of electrostatic potential (ESP) of "half-mustard type" phenothiazines [**7-12**] suggests that the ESP surface of urea site is important for the interaction between "half-mustard type" phenothiazines [**7-12**] and target cancer cell structures (or DNA base sequence). Actually, the urea site of "half-mustard type" phenothiazines displayed extensive variability in the energy of lone pair orbital and net atomic charges of N1, O and N3 atoms.*

Previous studies have shown a possible relationship between antitumor activity and dipole moments of "half-mustard type" phenothiazines (1, 2, 3, 4). NCI-Information Intensive-Approach (5) demonstrated that six "half-mustard type" phenothiazines [**7-12**] were more cytotoxic than six phthalimide compounds [**1-6**]. Among 54 tumor cell lines, colon-cancer cell was the most sensitive to six "half-mustard type" phenothiazines [**7-12**], followed by leukemia, melanoma, prostate-, CNS-, breast-, lung-, renal and ovarian cancer cells. Based on the experimental results, the significance of urea site on the electronically flexible phenothiazine framework was predicted. Therefore, the purpose of the present study was to investigate the possible relationship between cytotoxic activity and electronic structure of the particular urea site. In a plausible approximation, the role of the unshared electron pair on the urea site in half-mustard phenothiazines [**7-12**] was estimated by multiple regression analysis.

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Materials and Methods

Synthesis of chemicals. All related phenothiazines [**1-12**] were prepared as described previously (6).

Assay for antitumor activity. The 50% growth inhibitory concentration (GI_{50}), 100% tumor growth inhibitory concentration (TGI) and 50% lethal concentration (LC_{50}) values of six "half-mustard type" phenothiazines [**7-12**] were determined by dose-response curve, using 4 leukemias, 9 non-small cell lung carcinomas, 7 colon cancers, 5 CNS-cancers, 8 melanomas, 6 ovarian cancers, 8 renal cancers, 1 prostate cancer, and 6 breast cancer cell lines (5). The mean values of GI_{50}, TGI and LC_{50} were calculated in each tumor group for the six tested compounds [**7-12**]. Their $\log_{10}$ values were converted to molar concentration using the previous data for compounds [**7-12**] (Table I).

Theoretical calculations. About 30 conformations each for six compounds were processed to geometric optimization by the Molecular Mechanics 3 (MM3) method. Among about 30 optimized structures for each molecule, two typical structures with both maximum and minimum dipole moments were selected. The molecular orbital for these two structures were calculated by the Parametric Method 3 (PM3). MM3 and PM3 calculations were performed with the application of Alchemy 2000 Program (7) and winMOPAC (8), respectively. For this calculation, an IBM Aptiva E47 personal computer was used.

Results and Discussion

Relationship between cytotoxic activity and dipole moments. Table I shows cytotoxic activity (as measured by GI_{50}, TGI and LC_{50}) of "half-mustard type" phenothiazines [**7-12**] against 9 cell lines (2, 9, 10). The dipole moment (μ) was used to determine the interaction between two molecules with different dipoles. Their μ values of "half-mustard type" phenothiazines [**7-12**] were calculated by the PM3 method. The magnitude of μ ranged from 1.05 to 3.71 D in [**7**]; 0.72 to 4.22 D in [**8**]; 0.85 to 5.55 D in [**9**]; 1.54 to 2.78 D in [**10**]; 2.89 to 6.56 D in [**11**]; and 1.52 to 5.77 D in [**12**], respectively (Table II). The maximum μ ranged from 2.78 to 6.56 D, and the minimum μ ranged from 0.72 to 2.89 D, respectively (Table II). Interestingly, among six compounds

Table I. *Cytotoxic activity of six 1-(2-chloroethyl)-3-(2-substituted-10H-phenothiazin-10-yl)alkylureas [**7-12**] against nine different groups of cancer cell lines*[1,2].

Compd	Cytotoxic activity								
	Leukemia (4)	Non-small cell lung (9)	Colon-cancer (7)	CNS-cancer (5)	Melanoma (8)	Ovarian cancer (6)	Renal cancer (8)	Prostate cancer (1)	Breast-cancer (6)
				GI_{50} ($\times 10^{-5}$M)					
7	1.06	1.44	1.06	1.37	1.32	1.39	1.11	1.07	1.46
8	0.48	0.76	0.40	0.87	0.59	1.02	0.74	0.54	0.60
9	0.19	0.40	0.20	0.26	0.20	0.56	0.47	0.25	0.37
10	0.16	0.25	0.14	0.20	0.17	0.31	0.28	0.21	0.21
11	0.19	0.35	0.21	0.14	0.24	0.62	0.49	0.26	0.26
12	0.16	0.29	0.17	0.20	0.18	0.39	0.29	0.25	0.26
				TGI ($\times 10^{-5}$M)					
7	2.91	2.81	2.39	2.72	2.64	2.72	1.07	2.24	2.77
8	1.77	2.11	1.13	2.21	1.76	2.39	2.02	1.82	1.74
9	0.45	1.07	0.46	0.80	0.40	1.81	1.30	1.20	0.85
10	0.42	0.64	0.28	0.52	0.32	1.18	0.71	0.81	0.50
11	0.52	0.97	0.48	0.85	0.57	1.93	1.37	1.35	0.81
12	0.43	0.79	0.36	0.59	0.33	1.50	0.95	1.20	0.71
				LC_{50} ($\times 10^{-5}$M)					
7	7.59	5.52	5.03	5.37	5.22	5.33	5.11	4.68	5.68
8	5.96	4.85	2.75	4.92	4.10	5.15	4.60	4.27	4.83
9	1.41	2.83	1.03	2.47	0.92	4.54	3.23	3.55	2.68
10	2.11	2.04	0.57	1.86	0.60	4.25	2.07	3.02	1.39
11	4.04	2.62	1.14	3.09	1.45	4.88	3.42	3.80	2.40
12	1.40	2.53	0.83	1.99	0.69	5.11	3.17	3.63	2.08

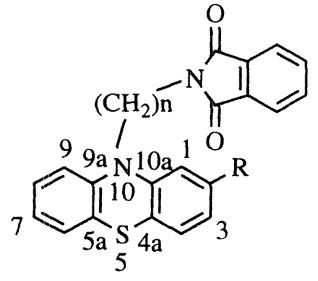
[1] ref.2. [2] Transformation: $10^{IA} \times 10^{5}$ = IB: in order to evaluate the outcome of the drug combination, fractional inhibitory concentration (FIC) indices were calculated as FICA + FICB, when FICA and FICB represent the minimum concentrations that drugs A and B inhibited the organism growth, respectively (10.11)

[**7-12**], compound [**11**] showed the highest μ value (Table I).

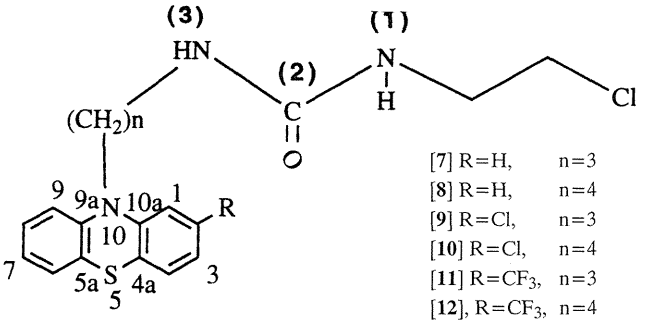
Relationship between cytotoxic activity and, π-HOMO or π-LUMO energy. If the cytotoxic activity of the compounds used in this study depends on the electron donor or electron acceptor property, then π-HOMO energy can be used as a measure of electron donating capacity. By this assumption, first, the π-HOMO energy of six compounds [**7-12**] was calculated: (-7.93 eV) [**9**], (-7.95 eV) [**8**], (-8.02 eV) [**7**], (-8.12 eV) [**10**], (-8.24 eV) [**12**] and (-8.27 eV) [**11**], respectively (Table II). Second, π-LUMO energies of six compounds [**7-12**] characterized by the electron acceptor property were calculated as follows: (-0.95 eV) [**12**], (-0.77 eV) [**11**], (-0.54 eV) [**10**], (-0.37 eV) [**9**], (-0.30 eV) [**7**] and (-0.24 eV) [**8**], respectively (Table II).

Assuming that the compound with the lower π-HOMO binds more easily to cancer cell DNA, compounds [**11, 12**]

Table II. *Dipole moment, molecular orbital energy and net atomic charge of "half-mustard type" phenothiazines [7-12], calculated by PM3 method.*



[1] R=H, n=3
[2] R=H, n=4
[3] R=Cl, n=3
[4] R=Cl, n=4
[5] R=CF_3, n=3
[6], R=CF_3, n=4



[7] R=H, n=3
[8] R=H, n=4
[9] R=Cl, n=3
[10] R=Cl, n=4
[11] R=CF_3, n=3
[12], R=CF_3, n=4

Compd's No	Dipole moment (in D)		Molecular orbital energy (in eV)						Net atomic charge	
	μ_{min} [a]	μ_{max} [b]	π-HOMO	π-LUMO	ΔE [c]	n_a [d]	n_b [e]	n_o [f]	C(=O)	O(=C)
7	1.05	3.71	-8.02	-0.30	7.72	-9.94	-9.72	-11.10	0.22	-0.40
8	0.72	4.22	-7.95	-0.24	7.71	-10.02	-9.87	-11.17	0.22	-0.40
9	0.85	5.55	-7.93	-0.37	7.56	-9.98	-9.65	-11.05	0.23	-0.41
10	1.54	2.78	-8.12	-0.54	7.58	-9.96	-9.66	-11.09	0.22	-0.41
11	2.89	6.56	-8.27	-0.77	7.50	-10.03	-9.84	-11.18	0.22	-0.39
12	1.52	5.77	-8.24	-0.95	7.29	-9.99	-9.67	-11.12	0.22	-0.40

[a] μ_{min}: minimum dipole moment. [b] μ_{max}: maximum dipole moment. [c] ΔE: energy gaps (HOMO-LUMO). [d] n_a: lone pair orbital of N1, O and N3 atoms. [e] n_b: lone pair orbital of N1 and N3 atoms. [f] n_o: lone pair orbital of O atom.

should have higher cytotoxic activity against these cells (Tables I and II). Actually, compound [**12**] (π-HOMO: -8.24 eV; π-LUMO: -0.95 eV), which had the lowest π-HOMO and π-LUMO energies, showed the highest cytotoxic activity against 9 cancer cell strains (Tables I and II). This suggests a possible electrochemical interaction between "half-mustard type" phenothiazines and cancer cell DNA.

Relationship between cytotoxic activity and orbital energies of lone pair of electron on urea site. The n_a orbital represents an unpaired electron orbital of N1, O and N3 atoms on urea site. The n_b and n_o orbitals represent an unpaired electron orbital at N1 and N3 atoms, and O atom on urea site, respectively (Table II).

Multiple regression analysis between cytotoxic activity and electronic structure in urea site. Among nine types of cancer cells, leukemia and colon cancer cells were the most sensitive to "half-mustard type" phenothiazines, based on GI_{50} values, possibly due to intra-molecular synergy between the antiproliferative property of phenothiazine and the leukemia specificity of "half-mustard type" phenothiazines. On the other hand, non-small cell lung, ovarian cancer and renal cancers were relatively resistant. In order to obtain a more quantitative correlation between cytotoxic activity and electronic property on urea site, the multiple correlation coefficient (r^2) and distribution function of F value between cytotoxic activity and various factors described above were calculated.

First, the multiple correlation coefficient (r^2) between GI_{50} values and four parameters of three orbital energies (n_a, n_b, n_o) and one net atomic charge of O atom in urea site were 0.91 for CNS-cancer cells and 0.98-0.99 for other 8 cancer cells. The F value (76.6) for colon-cancer was the highest, followed by renal cancer (F: 41.5), breast cancer (F: 22.9), melanoma (F: 19.6), non-small cell lung (F: 11.9), prostate cancer cells (F: 11.7), ovarian cancer cells (F: 10.9) and CNS cancer (F: 2.5). An acceptable correlation was not established, since F values of nine cancer cells for this model were much smaller than the five percent critical value of F (4, 1; 0.05) = 225.

Second, the multiple correlation coefficients (r^2) between GI_{50} values and four electronic parameters such

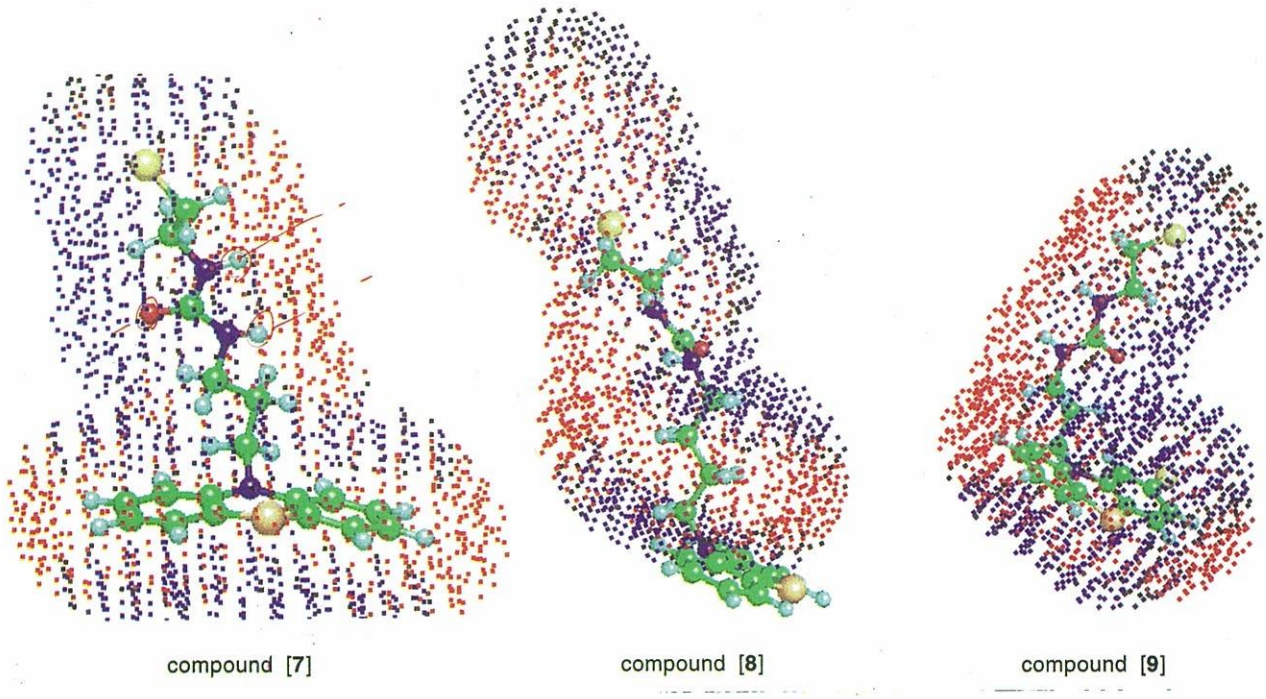


Figure 1. *Distribution of electrostatic potential of "half-mustard type" phenothiazines [7-9].*

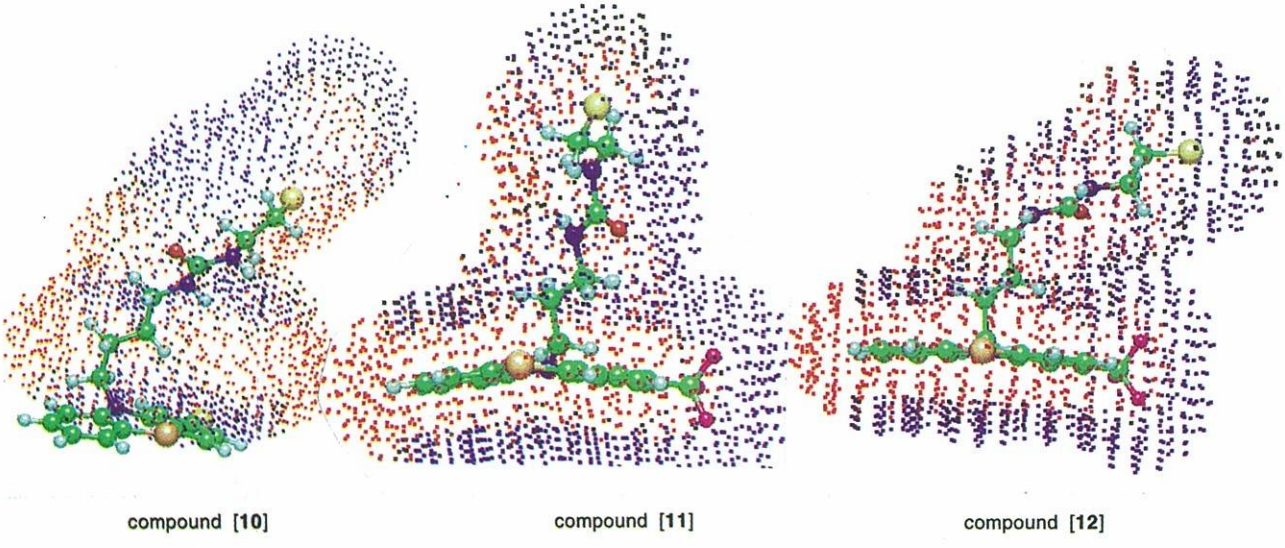


Figure 1 (cont.). *Distribution of electrostatic potential of "half-mustard type" phenothiazines [10-12].*

as orbital energies (n_a, n_o) and net atomic charges of C and O atoms on urea site were 0.98 for CNS-cancer cells and 0.99 for other 8 cancer cells. The F value for breast cancer cell (360683.6) was the highest, followed by melanoma (65965.5), leukemia (10632.5), ovarian cancer cell (1421.0), non-small cell lung (1362.3), prostate cancer (221.6), colon cancer (208.1), renal cancer (36.8) and CNS cancer cells (13.2). Thus, acceptable correlation was established, since the high F values of most of the cells were higher than the 5% critical value of F (4, 1; 0.05) = 225.

Third, based on TGI values, colon-cancer and melanoma were more sensitive than ovarian cancer, renal cancer and prostate cancer cells. The multiple correlation coefficient (r^2) between TGI values and four electronic parameters such as orbital energies (n_a, n_o) and net atomic charges of C and O atoms in urea site were 0.98 for renal cancer cell and 0.99 for other 8 cancer cells. The F value for colon-cancer cell (11801.3) was the highest, followed by ovarian cancer (662.6), melanoma (61.6), breast cancer (51.7), leukemia (39.9), non-small cell lung (33.5), CNS-cancer (25.0), prostate cancer (24.4) and renal cancer cells (13.6). Only the F value of colon cancer was significantly higher than 5% critical value of F (4, 1; 0.05) = 225. From the above calculations of r^2 and F values, colon cancer was found to be the most sensitive among 9 cancer cells to "half-mustard type" phenothiazines [**7-12**]. The inhibition of the drug transporter protein responsible for drug efflux might accumulate the "half-mustard phenothiazines" in the cancer cells, producing the cytotoxic activity against the multidrug resistant (MDR) cancer or colon cancer (6, 9).

Distribution of the electrostatic potentials. The ESP calculation of "half-mustard type" phenothiazines [**7-12**] were performed by PM3 method with ESP algorithm (10, 11). The ESP surface in the urea site might have an important role for the interaction between "half-mustard type" phenothiazines [**7-12**] and cancer cell surfaces or DNA (alkylation or intercalation) (Figure 1). Actually, a urea portion of "half-mustard type" phenothiazines has a considerably variable energy of the lone pair orbital and net atomic charges at N1, O and N3 atoms (Table II) (Figure 1). The region surrounding carbonyl oxygen atom has negative charge (-: symbol in blue of Figure 1), whereas the environment around the urea's NH at the opposite side has positive charge (+: symbol in red of Figure 1). These positive and negative charges might contribute to a complex formation *via* the dipole-dipole interaction, when a DNA component (*e.g.*, cytosine) can be attacked by the urea site of a "half-mustard type" phenothiazine.

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